Analysis of Garlic Cultivars Using Head Space Solid Phase Microextraction/Gas Chromatography/Mass Spectroscopy

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Abstract: Garlic has been widely used throughout history as a food additive for both its flavor and medicinal effects. The actual sulfide compounds found in garlic as well as the potential health benefits associated with garlic have been extensively studied. It has been shown that garlic preparation, growing conditions and cooking techniques, have a profound effect on the compounds present and the medicinal qualities afforded. Considering the number of garlic cultivars available and the large focus on growing food organically, differentiation between different garlic cultivars would be useful. Using SPME and GC-MS the sulfur compounds present in a variety of garlic types where identified and quantified. Principal component analysis enabled the differentiation of the cultivars studied and in one case the differentiation of Organic and Non-Organically grown soft neck garlic.

Keywords: Allium sativum, Garlic, Organic, Principal Component Analysis (PCA), Solid Phase Microextraction Gas Chromatography Mass Spectroscopy (SPME/GC/MS), Cultivar.

1. INTRODUCTION

Garlic (*Allium sativum*) has been utilized throughout history, serving both as a food supplement as well as a treatment for many ailments. The earliest records of garlic use date back to around 3,000 BC, when the father of Ayurvedic medicine, Charak, stated that garlic helped maintain fluidity of blood and strengthened the heart [1]. Recent research has revealed many different benefits of garlic on human health, including anticarcinogenic, antifungal, and antibacterial properties [2]. In animal studies garlic was found to modulate the activity of proteins that are linked with diabetes and obesity-related cardiac disorders [3].

It is these benefits that have driven the extensive research on the volatile components of garlic to determine which compounds are responsible for the health benefits. Many specific chemicals in garlic have been directly linked to having an inhibitory effect on chemical carcinogenesis [4]. However a problem faced when working with garlic is the instability of the sulfur-containing compounds. The amino acid alliin is present in whole garlic cloves.

When the clove is crushed, the enzyme alliinase (Scheme **1**) is activated and breaks down alliin into allicin and other allyl thiosulfinates [5]. At least 35 different compounds have been identified in garlic [6]. These sulfur-containing compounds have been the focus of the many quantitative and qualitative studies on garlic and related species [7].

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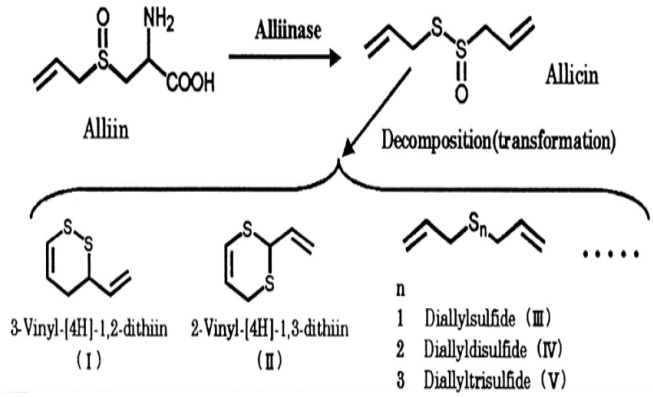

Scheme 1. Breakdown of the amino acid alliin into allicin by the enzyme alliinase upon the crushing of a garlic clove. Allicin subsequently decomposes into different sulfide compounds, the main components of garlic [16].

The allicins and sulfide compounds in *Allium* plants, notably garlic, onion (*A. cepa*), and scallion (*A. ascalonicum*), are presumed to be responsible for their distinct flavors and aromas [8]. Studies have shown the chemical distinctions between the species in the amount and types of compounds present [7, 8]. Through Principal Component Analysis (PCA), differentiating garlic has been performed based on diversity in garlic genes [9], the country of origin [10], the type of food preparation technique [11], and morphological characteristics of garlic [12]. To our knowledge however, there is no published data on the use of PCA to separate garlic cultivars.

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The means by which garlic is grown and later prepared may also have a significant effect on the sulfide compounds present and hence on the benefits available to the consumer. Garlic grown in a cooler climate has a different distribution of compounds versus garlic grown in a warmer climate [13]. The way garlic is prepared, whether it be deep-fried, microwave-heated, oven-baked, etc., also has an effect on its chemical composition [14]. The health benefits can also vary with preparation. Freshly cut garlic has been found to have greater cardio protective effects, compared to processed garlic [3].

2. MATERIALS

2.1. Garlic Samples

Non-Organic (Soft Neck, Spice World Inc. Orlando FL), Organic (Soft Neck, Spice World Inc. Orlando FL) and Elephant Garlic (*A. ampeloprasum)* (Spice World Inc, Orlando FL) where purchased from Wegmans Supermarket (Erie, PA, USA). Elephant garlic is not a true garlic, but is more closely related to leeks. The two other garlic cultivars used in this study were 'Shvelisi' and 'Dailey'. Shvelisi is a purple stripe cultivar also known as 'Chesnok Red' in the trade. Dailey is a popular locally adapted cultivar that is most likely a Rocambole type. These two cultivars were grown in an organic garden in Erie, PA., and harvested in July, 2010.

2.2. Instrumentation

Analysis of garlic samples was performed using an Agilent Technologies 7890A Gas Chromatograph equipped with a 5975C Mass Selector and Gerstel MPS2 (Multi- Purpose Autosampler). The column used in the GC was a DB-624, 30 m x 0.250 mm x 1.4 µm with a carrier gas of Helium. MSD ChemStation Software (Version E.02.01.1177) was used for peak analysis of the volatile components. The Unscrambler X (CAMO Software) was used for PCA analysis.

3. METHODOLOGY

3.1. Sample Preparation

All garlic samples were prepared in the same manner. The garlic bulb was broken apart into individual cloves, then the cloves were peeled so no outer covering remained. The ends of the cloves were cut off, then the remaining garlic was chopped into similar-sized pieces using a 3 in. kitchen knife (EKO Stainless, USA). Exactly 2.0 g of the garlic was measured on a balance (Adventurer Pro, model AV114, Ohaus). Three samples were collected from three separate bulbs of each of the garlic cultivars.

3.2. Sample Storage

Garlic samples were immediately placed into 20 mL clear screw cap vials (Gerstel Inc.), weighed and the vials sealed with magnetic screw caps lined with blue silicone/PTFE septa (Gerstel Inc.). Experimental results showed that leaving garlic uncovered over any period of time significantly reduced the amount of volatiles present.

3.3. SPME Technique and Instrument Parameters

Garlic sample volatiles were extracted for 3.0 min at room temperature using Solid Phase Microextraaction (SPME). This technique is ideally suited for solid samples [15]. A Carboxen Polydimethylsiloxane (CAR/PDMS) SPME fiber was chosen for optimal volatile extraction, based on published research [17, 18]. Desorption (5.0 min), took place in the GC injection port operating in split mode (20:1) at a temperature of 250 °C. The carrier gas (Helium) flow rate was set at 1 mL/min with a temperature program of 50 °C for 3 min, then 5 °C/min to 210 °C for a total run time of 35.0 min. Scan parameters were set from 15.0 to 240.0 amu for the Mass Spectrometer analysis.

Absorption time was optimized as shown in Fig. (**1**). using a sample of nonorganic garlic. A three minute extraction time gave maximum total volatiles.

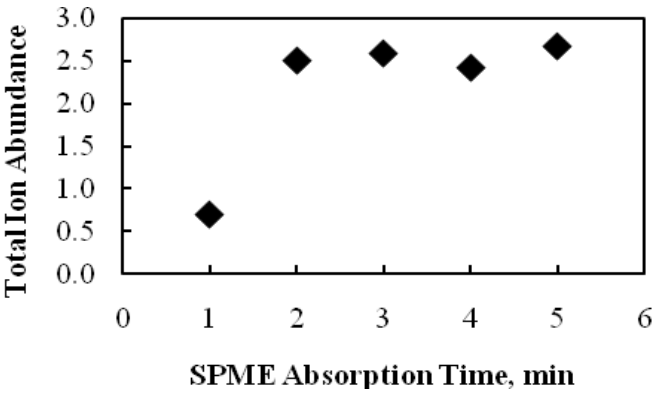

Fig. (1). Total volatiles extracted (area from total ion chromatogram $x10^9$) versus SPME extraction time for a two gram sample of nonorganic garlic.

Fiber desorption time was set to five minutes. Using this desorption time no analyte carryover could be detected in a blank sample analyzed after prior absorption of garlic analytes followed by GCMS analysis.

3.4. Principal Component Analysis Optimization

A consistent, reproducible method for sample preparation and volatile extraction that enabled a successful PCA separation was obtained by using SPME extraction and a 7890A Gas Chromatogram equipped with an Autosampler. When the five different garlic samples (see "Garlic Samples for SPME and PCA Analysis") were initially analyzed using PCA, no separation was seen. Upon further examination of the chromatograms, it was determined that the garlic samples were being analyzed too quickly after preparation. Compound levels where changing during the analysis, limiting the reproducibility of the samples run in triplicate. Separation through the PCA was not seen using these samples. Chromatograms showing this progression are shown in Fig (**2**).

To solve this problem the garlic samples were allowed to sit in a sealed vial for one, seven, and fourteen days, to allow volatile composition to stabilize. Three separate trials for each time interval were performed to optimize separation when analyzed with PCA. The 14 day samples showed the best separation in the PCA analysis.

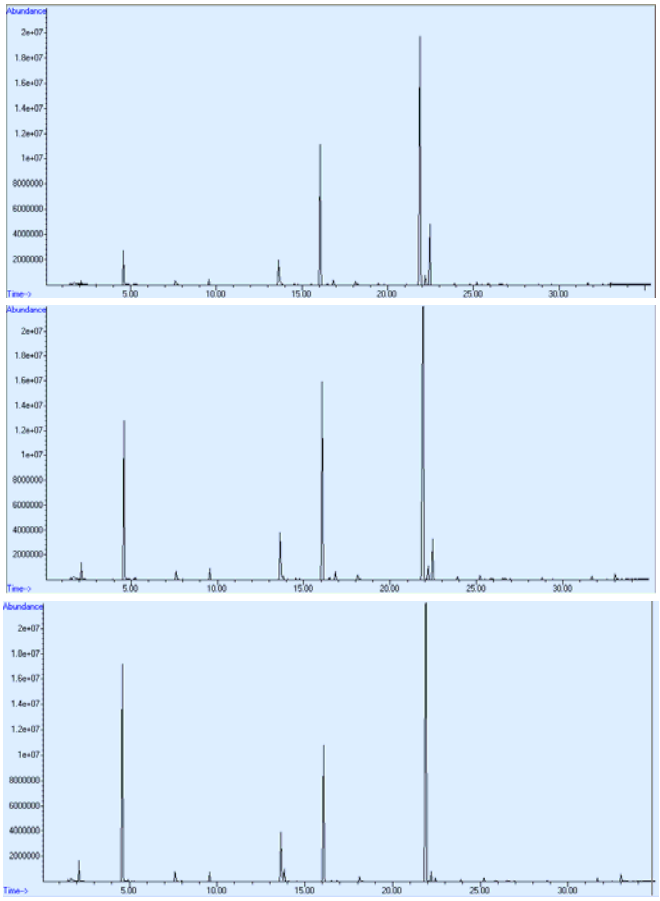

Fig. (2). Three chromatograms of the three freshly cut sample of Shvelisi garlic. Top – analysis within 5 min. Middle – after standing for 35.0 min. Bottom - after standing for 70.0 min.

The change in volatile composition of fresh cut garlic is not surprising considering the process by which garlic sulfur compounds are formed [5].

4. RESULTS AND DISCUSSION

Table **1** shows quantitative differences between the five different garlic samples. The total ion chromatograms (total of the relative abundances) for each of the eight volatiles measured is listed along with the name of the actual compound measured. It can be seen that Nonorganic garlic had the least amount of volatiles present, whereas Elephant garlic had the most. These values may help to explain why the odor or taste of some garlic cultivars may be perceived as "strong" while others are perceived as "mild".

Fig. (**3**) shows the results of Principal Component Analysis on the garlic samples analyzed. “Facter 1” and “Facter 2” have been used in place of Principal Component 1 (PC-1) and Principal Component 2 (PC-2) [19] since "Principal Components" are in fact not single compounds but a mathematical relationship between all of the compounds in the samples analyzed [20]. Although a number of PC's can be calculated only two PC-1 and PC-2 were required to explain most of data variance. PC-1 and PC-2 accounted for 98% of the total variance (75% for PC-1 and 23% for PC-2).

In the same fashion PCA has recently been used to separate six species of blueberries [21].

5. CONCLUSION

After sample preparation and analysis were optimized, known cultivars of garlic, and non-organically grown garlic were distinguished using Principal Component Analysis.

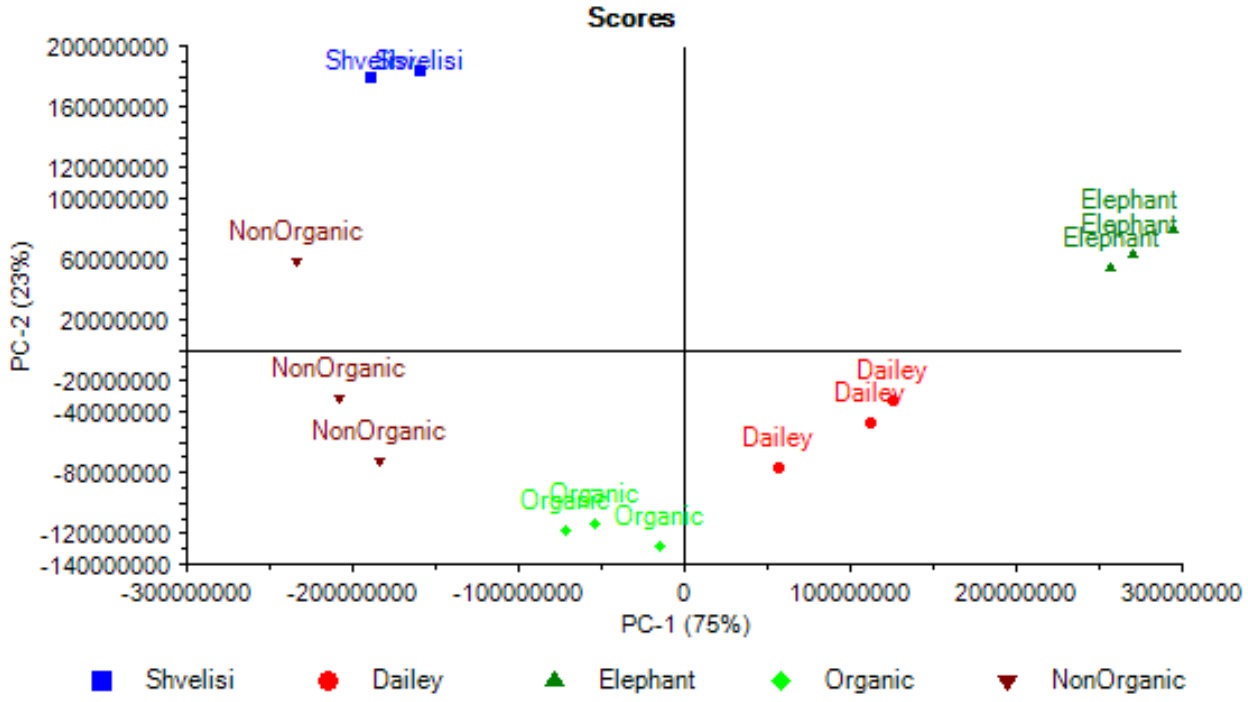

Fig. (3). Principal component analysis (PCA) of garlic samples**.**

Table 1. Relative Amounts[b] of Major Analytes in Garlic

Compound[a]	Shvelisi	Dailey	Elephant	Organic	Nonorganic
Methanethiol	25.1	2.0	2.9	1.9	6.3
Methyl Thirane	345.1	2.9	2.7	33.5	276.3
Allyl Methyl Sulfide	47.5	42.7	214.2	9.1	5.7
Dimethyl Disulfide	9.7	4.9	48.5	2.3	2.6
Allyl Sulfide	110.6	259.5	388.7	57.2	46.2
Methyl 2-Propenyl Disulfide	173.8	145.9	374.4	119.9	35.2
Diallyl Disulfide	569.0	1,458.9	1,434.1	648.5	268.7
Allyl Trisulfide	1.9	52.4	41.5	15.4	0.7
Total	1282.7	1969.2	2507.0	887.8	641.7

[a]Compound identification based on mass spectrum [22] and retention index matching to those of commercially available reference samples or literature values. [b]Average of triplicate analysis expressed as the total ion abundance (TIC) for each compound ($x10^6$) when 2.0 g of each garlic type was analyzed.

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